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ABSTRACT

In the interest of learning more about the evolution and species specificity of the relatively obscure autotrophic bacteria, the authors studied the nucleic acid composition of five bacterial species of the pseudomonadales order, including: one autotrophic green sulfur photosynthesizing; one purple sulfur photosynthesizing; one purple nonsulfur phototrophic; two colorless sulfur species. The taxonomic basis for the study was the nucleotide constituency of the desoxyribonucleic acid (DNA) in the microorganisms, as well as their morphological and physiological attributes. The evidence indicates a phylogenetic relationship between the green photosynthesizing bacteria, some colorless sulfur bacteria, azotobacters, and the blue-green algae, as well as a common heredity linking these organisms. Other interrelationships, including those with certain heterotrophic species, possible common origins, and favorable directions of evolution are discussed in light of DNA structure.

Of all the various microorganisms, the heterotrophic bacteria have been subjected to the most study up to the present with regard to their nucleic acid composition. The nucleotide composition of the desoxyribonucleic acid (DNA) of these bacteria is associated with their hereitary characteristics and their species idiosyncrasies. With regard to the autotrophic bacteria, although they have been inadequately studied up to now, the scant findings nevertheless clearly indicate that their DNA composition possesses a certain species specificity (refs. 1 to 3). In this connection, it is believed that an extensive study of DNA composition specificity in autotrophic bacteria will help to answer some questions about their evolution and taxonomy. In addition, nucleic acid research of this interesting group of organisms is acquiring special meaning and importance in connection with the evolution of such functions as photosynthesis, chemosynthesis, nitrogen fixation, etc.

We have studied the nucleotide composition of DNA in five species of bacteria of the pseudomonadales order (ref. 4): the autotrophic green sulfur photosynthesizing bacteria chloropseudomonas ethylicum (chlorobacteriaceae family); the purple sulfur photosynthesizing, bacteria rhodopseudomonas sp., which are capable of autotrophic growth; the purple nonsulfur phototrophic bacteria rhodospirillum rubrum (athiorhodaceae family); and the two species of colorless sulfur bacteria; thiobacillus thioparus and thiobacillus ferro-oxidans (both of the thiobacteriaceae family)[1].

Chloropseudomonas ethylicum represents a new species of bacteria, recently isolated in a pure culture in the Microbiology Department of Moscow University (ref. 5). This organism, in contrast with its related green counterpart, the highly autotrophic bacterium chlorobium thiosulfatophilum, has the ability to assimilate ethanol as a source of carbon supply. To study the composition of DNA, we used a culture of Ch. ethylicum, grown in light at 30° in a modified Larsen's mineral medium (refs. 5 and 6), as well as a culture grown in the same medium with the addition of 0.1% ethanol. Rhodopseudomonas sp. was grown in Larsen's medium (ref. 6) with 0.5% sodium acetate added, and rhodospirillum rubrum in Molisch's medium with peptone (ref. 7). The culture of thiobacillus thioparus was grown in a fermenter at 30° for 60 hours in 85 liters of Vishniac's medium (ref. 8), aerated in an atmosphere with 2 to 5% CO_2 content. The thiobacillus ferroxidans culture was grown in the fermenter for 60 hours in 100 liters of Beck's medium (ref. 9) (pH 3.0) aerated by atmosphere with periodic addition of 1 - 2% CO_2. The composition of the DNA was determined by an earlier method (ref 10).

The data we obtained on the DNA composition of certain photosynthesizing and thionic bacteria is presented in Table 1. These data are averages of from

TABLE 1

NUCLEOTIDE COMPOSITION OF DNA OF CERTAIN PHOTOSYNTHESIZING AND THIONIC BACTERIA

Organism	BASE, MOLAR %				$\frac{pur}{pyr}$	$\frac{G+T}{A+C}$	$\frac{G+C}{A+T}$
	G	A	C	T			
Chloropseudomonas ethylicum *	28,8	21,2	28,8	21,2	1,00	1,00	1,36
Chloropseudomonas ethylicum **	28,6	21,6	28,6	21,2	1,01	0,99	1,34
Rhodopseudomonas sp.	32,0	18,2	32,0	17,8	1,01	0,99	1,78
Rhodospirillum rubrum	31,7	18,4	31,7	18,2	1,00	1,00	1,73
Thiobacillus thioparus	29,5	21,6	29,7	19,2	1,05	0,95	1,45
Thiobacillus ferrooxidans	30,3	20,6	28,5	20,6	1,04	1,04	1,43

* Grown in mineral medium.

** Grown in same substrate with the addition of ethanol.

[1]We express our gratitude to S.I. Kuznetsov for supplying us with a culture of thionic bacteria.

2 to 5 separate determinations from not less than two parallel hydrolysates. The r.m.s. deviation from the average base molar percentages shown in table 1 was about 1 or 2%.

Corresponding with the relations established by Chargraff, the ratios pur/pyr (purine to pyrimidine) and (G+T)/(A+C)(guanine + thymine to adenylic acid + cytidine) are almost unity for the DNA of all the organisms studied. The DNA of the investigated bacteria is of the GC type.

The nucleotide composition of the DNA cells of green photosynthesizing bacteria, grown in a mineral medium with or without the addition of ethanol, is practically the same. This indicates that the DNA composition of Ch. ethylicum does not depend on the nature of its carbon supply. Ch. ethylicum and the green photosynthesizing bacterium Ch. thiosulfatophilum which was previously studied by us (ref. 1) have identical DNA compositions. This again confirms the assumption of genetic and taxonomic similarity between these two species of bacteria of the chlorobacteriaceae family (ref. 11). The green bacterium studied in the present investigation is regarded as belonging to genus chloropseudomonas (ref. 5) because of a certain morphological similarity to the pseudomonodales, but in respect to DNA composition this organism is basically different from both the typical nonphotosynthesizing and the photosynthesizing purple pseudomonodales. The green bacteria differ sharply in DNA composition from the purple photosynthesizing bacteria; the DNA of the latter belongs to the relatively high GC type.

Significant differences in DNA composition were not found in the purple sulfur and nonsulfur photosynthesizing bacteria that we investigated. Furthermore, these bacteria were found to be very similar in DNA composition to the sulfur purple photosynthesizing bacteria chromatium sp. that we had previously studied,(G+C)/ (A+T) = 1.76) (ref. 1). Evidently, this similarity in DNA composition is not accidental and reflects the genetic closeness of the sulfur and nonsulfur purple bacteria. In this connection, it should be recalled that many microbiologists consider the division of purple bacteria into two families, thio- and athiorhodaceae, as distincly arbitrary. In spite of the fact that representatives of various genera of purple bacteria are highly similar in DNA composition, this evidently does not exclude the possibility of the existance of specific slight variations, at least within the confines of a few genera. So, for example, one of the most heterotrophic genera of purple bacteria, rhodopseudomonas spheroides (ref. 12) (G+C)/(A+T) = 2.08), differs in DNA composition from the rhodopseudomonas sp. that we studied (G+C)/(A+T) = 1.78). Thus, distinct species differences in DNA composition can show up among the representatives of the genus rhodopseudomonas. These slight variations in the purple bacteria evidently represent a certain reflection of the natural process of mutation. The marked similarity in DNA composition and the similarity of many morphological-physiological characteristics are a convincing indication that the purple photosynthesizing bacteria represent a group of organisms that have a close phylogenetic relationship.

It is interesting that the purple bacteria are very close in DNA composition to the typical heterotrophic bacteria of the pseudomonas genus, since the indicator of DNA composition specificity, i.e., the value of the ratio

(G+C)/(A+T), in pseudomonas (ref. 13) varies approximately between the same limits (1.7 to 2.08). To a certain extent, this can indicate a phylogenetic kinship of the photosynthesizing purple bacteria with the typical heterotrophic species of the pseudomonadaceae family. In this connection, it is logical to assume that contemporary purple bacteria and heterotrophic pseudomonas could have originated from a single heterotrophic predecessor. At the same time it is also possible that the anaerobic pseudomonas gave rise to the purple bacteria.

The question of the phylogenetic relationship of the purple and green bacteria is still being debated. Some think that evolution proceeded from the green to the purple bacteria (ref. 14) and others are of the opinion that the green bacteria originated from the purple (ref. 15). On the strength of a number of more primitive physiological features of the purple bacteria, the first-mentioned mode of evolution seems less probable. However, from our point of view, neither does the second mode of evolution appear so self-evident. The green and purple bacteria indeed possess a number of common features, but the green bacteria differ sharply from the purple in DNA composition and the nature of their photoactive pigments (ref. 16); they are more autotrophic and aerobic than the purple bacteria (ref. 11), etc. Without denying the possibility of evolution from the purple to the green bacteria, it is still possible to assume that the green bacteria could have originated from some heterotrophic predecessor that was comparatively close to the forerunner of the purple bacteria but which possessed slight different DNA composition. Theoretically, such a predecessor of the green bacteria could obviously have been an organism that was to a certain extent similar to aerobacter aerogenes (G+C)/(A+T) = 1.31 (ref. 13), a nitrogen-fixing facultative aerobic.

According to the notions of some authors, the photosynthesizing bacteria have a definite phylogenetic relation to the thionic bacteria (refs. 14, 17, and 18). In this regard, it is of interest that the sulfur and iron-oxidizing thionic bacteria studied by us are actually similar in DNA composition to the green sulfur photosynthesizing bacteria. Furthermore, these thionic bacteria, as species of one genus, were found to be similar to one another in DNA composition. In the opinion of Burris (ref. 14), the desulfovibrio group of desulfurizing bacteria could also have originated as a secondary branch of the photosynthesizing bacteria. In DNA composition, the asporogenic, halophile desulfovibrio (ref. 3) is close to the green sulfur bacteria, but the anhalophile desulfovibiro is close to the purple bacteria. The findings on DNA composition do not thereby contradict the assumption that the photosynthesizing bacteria could have been the forerunners of desulfovibrio. At the same time the photosynthesizing bacteria have nothing in common in regard to DNA composition with the sporogenic desulfovibrio (ref. 3). This is evidently explained by the fact that desulfovibrio represents a miscellaneous group of microorganisms united by a certain physiological characteristic.

The notion has been advanced (ref. 17) that photosynthesizing bacteria, following loss of certain pigments, could have given rise to such aerobic nonsymbiotic species as the azotobacter. In the light of findings on DNA composition, this assumption seems quite plausible. The DNA of azotobacter is especially close to the DNA of the green bacteria. In this regard, it is interesting to note that the closest form to the green bacteria with respect to

DNA composition is the most pigmented species A. chroococcum (G+C)/(A+T) = 1.35, while the less pigmented species A. vinelandii and A. agile (G+C)/(A+T) = 1.22, are not so close to the green bacteria (ref. 19). At the same time, the opinion exists that the most likely precursor of azotobacter was aerobacter aerogenes (ref. 14). Furthermore, some authors think that azotobacter is considerably closer in the evolution system to the blue-green algae than to the true bacteria (ref. 20). The impression is thus formed that azotobacter on the one hand is close to the green photosynthesizing bacteria and on the other hand, that it is close to the blue-green algae.

A number of authors are strongly convinced that the photosynthesizing bacteria are the evolutionary predecessors of the blue-green algae (refs. 14 and 15). Of all the photosynthesizing bacteria, the green bacteria most nearly approach blue-green algae with regard to their DNA composition (ref. 21). Thus, closeness in respect to DNA composition and similarity of other important physiological-biochemical indications testify in favor of the well-known versions of the phylogenetic connection between the green photosynthesizing bacteria, certain colorless sulfur bacteria, azotobacters, and the blue-green algae. However, the question still remains open whether in fact the colorless sulfur bacteria and the azotobacter originated directly from the photosynthesizing bacteria with a resultant loss of their photosynthesizing function, or whether in fact the photosynthesizing bacteria are the direct predecessors of the blue-green algae. The only certain thing is that each of these groups of organisms has gone through its own protracted evolution and, most probably, that they are all linked by a common origination from some single heterotrophic and anaerobic ancestor.

It should be mentioned in conclusion, that the evolution of the photosynthesizing organism (purple and green bacteria, blue-green algae) is reflected, so to speak, in the structures of their nucleic acids. This evolution is definitely accompanied by a decreasing content of GC-pairs and increasing content of AT-pairs of bases in the DNA composition. The purple bacteria, most ancient and primitive of all the photosynthesizing bacteria, are characterized by the highest GC-type of DNA. The green bacteria possess DNA of less pronounced GC-type. In the blue-green algae, which have attained the highest degree of evolution with respect to nitrogen fixation and possess quite perfect photosynthesis, similar to the photosynthesis of the highest plants, the (G+C)/(A+T) ratio of its DNA is still smaller than in the green bacteria. Moreover, in the blue-green algae there is even DNA of the AT type, which is characteristic of all higher plants. The evolution of green algae was also accompanied by a decrease of the (G+C)/(A+T)ratio in their DNA (ref. 21).

The relation of DNA structure to evolution indicates that further investigation of these genetically significant compounds and, particularly, of their nucleotide sequences, will more distinctly reveal phylogenetic interrelations among the various groups of photosynthesizing organisms.

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